UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

9/23/1997

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Imidacloprid - Review of Domestic Animal Safety Studies with Advantage™ Spot-On Formulation

DP Barcodes: D229579, D232036
PC Code: 129099
Case: 005593, 005596
Submission: S511142, S515745

FROM: Virginia A. Dobozy, V.M.D., M.P.H., Veterinary Medical Officer
Reregistration Review Branch I Virginia A Dobozy 9/23/97
Health Effects Division (7509C)

TO: Tina Levine/Elizabeth Haeberer/ PM 4
Registration Division (7505C)

THRU: Whang Phang, Ph.D., Branch Senior Scientist Wh Phang 9/23/97
Reregistration Review Branch I
Health Effects Division (7509C)

Action Requested: Review domestic animal safety studies in kittens and puppies with Advantage™ (9.1% imidacloprid) spot-on formulation.

Recommendation: Reregistration Review Branch I has completed review of two domestic animal safety studies in kittens and one study in puppies. One of the studies in kittens is acceptable; the puppy study is acceptable. The unacceptable study in kittens (MRID No. 44157301) demonstrated that 6 week-old kittens stressed from weaning cannot tolerate 5X the recommended dose of Advantage™. The acceptable study in kittens (MRID No. 44157302) demonstrated that 8 week-old kittens can tolerate a 5X the recommended dose of Advantage™. The acceptable study in puppies (MRID No. 44099801) demonstrated that 7 week-old puppies can tolerate a 5X the recommended dose of Advantage™. See further recommendations under Incident Data, Labeling and Promotional Literature .



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A summary of each study is presented below; the complete DERs are attached.

DATA REVIEW

1. MRID No. 44157301 - "General Safety Evaluation for Topical Use of Imidacloprid (Advantage™) Spot-On on Six Week Old Kittens.

Material Tested: Advantage™ (9.1% Imidacloprid)

EXECUTIVE SUMMARY: In a domestic animal safety study (MRID # 44157301), six 6 week-old kittens/sex were treated with Advantage™ (9.1% imidacloprid) at 5X the recommended use rate (2.0 ml). Six kittens/sex were also treated with the vehicle control at the recommended use rate (0.4 ml). According to the study protocol, the animals were supposed to receive 8 treatments at weekly intervals. However, two males and two females in the imidacloprid-treated group died or were euthanized within 72 hours after the first treatment. On necropsy, the two females had suppurative cholangiohepatitis which was assumed to be due to an ascending bacterial infection in the liver. In addition, one female had mild diffuse hepatic lipidosis. There were no remarkable findings in the males. The study protocol was revised to test the toxicity of the major vehicle excipient. Three six week-old female kittens were treated with the vehicle at 5X the recommended use rate. All three died within 24 hours of treatment. The study report concluded that the kittens were stressed from weaning and were not able to tolerate 5X the recommended use rate.

The study is considered **unacceptable** and **cannot be upgraded**. It was terminated prior to completion due to animal welfare considerations.

2. MRID No. 44157032 - "General Safety Evaluation for Topical Use of Imidacloprid (Advantage™) Spot-On on Kittens Eight Weeks of Age

Material Tested: Advantage™ (9.1% Imidacloprid)

EXECUTIVE SUMMARY: In a domestic animal safety study (MRID # 44157302), six 8 week-old kittens/sex were treated with Advantage™ (9.1% imidacloprid) at 5X the recommended use rate (2.0 ml) at weekly intervals for eight treatments. Six kittens/sex were treated with the vehicle control at the recommended use rate (0.4 ml) at weekly intervals for eight treatments. There was no evidence of treatment-related toxicity in clinical signs or clinical pathology parameters. All animals gained weight during the study. It was demonstrated that 8 week-old kittens can tolerate a dose of 5X the recommended use rate.

The study is considered **acceptable** and **satisfies the draft guideline requirements (81-6) for a domestic animal safety study.**

3. MRID No. 44099801 - "General Safety Evaluation for Topical Use of Imidacloprid (Advantage™) Spot-On on Puppies"

Material Tested: Advantage™ (9.1% Imidacloprid)
EXECUTIVE SUMMARY: In a domestic animal safety study (MRID # 44099801), six 7 week-old puppies/sex were treated with Advantage™ (9.1% imidacloprid) at 5X the recommended use rate (2.0 ml if < 10 lbs; 5.0 ml if > 10 lbs) at weekly intervals for eight treatments. Six puppies/sex were treated with the vehicle control at the recommended use rate (0.4 ml if < 10 lbs; 1.0 ml if > 10 lbs) at weekly intervals for eight treatments. There was no evidence of treatment-related toxicity in clinical signs or clinical pathology parameters. All animals gained weight during the study. It was demonstrated that 7 week-old puppies can tolerate a dose of 5X the recommended use rate.

The study is considered **acceptable** and **satisfies the draft guideline requirements (81-6) for a domestic animal safety study.**

INCIDENT DATA

At a June 11, 1997 meeting of the 6(a)(2) Team, the issue of incidents of adverse reactions with Advantage™ was discussed. A cursory review of all incidents reported to the Incident Data System as of May 15, 1997 was presented by this reviewer. The incident reports from the registrant are very sketchy and disorganized. However, based on the data which could be extracted from the reports, a rough estimate of the number of animals involved per effect is listed below. An animal was only counted once and was placed in the most severe category. For example, an animal which died was not counted in another category, even though it may have had other signs prior to death.

| Effect | Dogs | Cats |
|---|---|---|
| Death | 17 | 46 |
| Skin irritation | 132 | 100 |
| CNS Signs (e.g. seizure) | 35 | 31 |
| General (e.g. lethargy) | 29 | 49 |
| G-I (e.g. vomiting) | 32 | 33 |
| Eye damage | 3 | 7 |

CNS = central nervous system; G-I = gastrointestinal system

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In some of the incident reports, species was not identified. The number of animals involved in these reports is listed below.

Death - 7
Skin irritation - 67
CNS Signs - 6
General - 12
G-I - 27
The total number of animals involved could have been as high as 633. Since May, there have been at least 52 reports for the five Advantage™ products (Reg. Nos. 11556-116 through 11556-120), according to the Incident Data System. Many of these reports are summaries so the exact number of animals involved cannot be determined without reviewing the actual incident reports in depth.

There are also reports of adverse effects in humans, mostly of a dermatological nature. Effects reported include rash/hives, blisters, puffy eyes, swelling of hand, feet or lips and tingling. Occasional upper respiratory and gastrointestinal effects have also been reported.

At the 6(a)(2) meeting, this reviewer suggested that the Registration Division contact the registrant to provide more detailed reports so that the exact nature and number of animals and humans involved in the incidents could be determined. It was decided that the pending domestic animal safety studies should be reviewed and evaluated before any further action was taken on the incident reports. It is the recommendation of this reviewer that the issue of incident reports should be addressed before labeling for use in younger animals is granted.

LABELING

Labels received from the Registration Division were reviewed; it is assumed that these are the latest versions. The following comments are offered:

1) Some of the labels do not contain the required language in PR Notice 96-6.

2) Advantage™ 9 dated 1/11/96 (Reg. No. 11556-116) for use on cats and kittens 9 lbs. and under

a. The November 8, 1996 letter from Bayer, which accompanied the kitten studies, states that this product is restricted for use on kittens younger than four months of age. However, this label states, "Do not use on kittens under 10 weeks of age."

b. The November letter states that retreatment is permitted if necessary (in case where shampooing could remove the previous treatment), however retreatment intervals at less than one week are prohibited. (This is justification for administering the product at weekly intervals in the kitten domestic animal safety study.) This label does not have any instructions about reapplication as do some of the other Advantage™ labels.

3) Advantage 10, 20 and 55 for use on dogs 10 lbs and under, 11-20 lbs and 21-55 lbs,

respectively (Reg. Nos.11556-117, -119 and -120, respectively) dated 10/18/96 all state "Do not use on puppies under four months of age". (No label for 11556-118 was supplied.)

4) Advantage 110 dated 1/11/96 (Reg. No. 11556-121) for use on dogs over 55 lbs.

a. This label states "Do not use on puppies under eight weeks of age".

b. This label states that reapplication after shampooing is recommended but no restriction on the interval is given.

5) Advantage 100 dated 10/18/96 (Reg. No. 11556-122) for use on dogs over 55 lbs.

a. According to these labels, there are two products for use on dogs over 55 lbs with different registration numbers.

PROMOTIONAL LITERATURE

Advantage™ products are heavily promoted to the veterinary profession. Much of the promotional literature does not contain the current age restrictions in kittens and puppies. In brochure B97109, under the heading "A Gentle Advantage", there is a discussion of the 6 week-old puppy and 8 week-old kitten domestic animal safety studies which were reviewed in this memo. There is no statement in this section of the brochure or any other part about the current age restrictions. Some of the promotional literature also contradicts the labeling efficacy information. Brochure B97110 states, "Independent studies show that Advantage keeps working even if your pet gets wet. So you can still bathe your dog and not worry about losing your flea protection." Most of the dog labels state that shampooing can shorten flea protection and there should be a reapplication of the product. Copies of these brochures are attached to this memo.

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Reviewed by: Virginia A. Dobozy, V.M.D., M.P.H. Virginia A Dobozy Date 9/17/97
Reregistration Review Branch I, Health Effects Division (7509C)
Secondary Reviewer: SanYvette Williams-Foy, D.V.M. S. Y. Williams-Foy Date 9/17/97
Registration Action Branch II, Health Effects Division (7509C)

# DATA EVALUATION REPORT

STUDY TYPE: Domestic Animal Safety Study/Kittens (86-1; OPPTS 870.7200)

DP Barcode: D232036
P. C. CODE: 129099
Submission Code: S515745
Tox. Chem. No.:

TEST MATERIAL (PURITY): Imidacloprid (Advantage™) (9.1%)

SYNONYMS:

CITATION: Schmidl, J. and R. Arther (1996) General Safety Evaluation for Topical Use of Imidacloprid (Advantage™) Spot-On on Six Week Old Kittens. DeSoto Research Facility. Report No. TR-96F-004, August 20, 1996. MRID 44157301. Unpublished.

SPONSOR: Bayer Corporation
Shawnee Mission, Kansas

EXECUTIVE SUMMARY: In a domestic animal safety study (MRID # 44157301), six 6 week-old kittens/sex were treated with Advantage™ (9.1% imidacloprid) at 5X the recommended use rate (2.0 ml). Six kittens/sex were also treated with the vehicle control at the recommended use rate (0.4 ml). According to the study protocol, the animals were supposed to receive 8 treatments at weekly intervals. However, two males and two females in the imidacloprid-treated group died or were euthanized within 72 hours after the first treatment. On necropsy, the two females had suppurative cholangiohepatitis which was assumed to be due to an ascending bacterial infection in the liver. In addition, one female had mild diffuse hepatic lipidosis. There were no remarkable findings in the males. The study protocol was revised to test the toxicity of the major vehicle excipient. Three six week-old female kittens were treated with the vehicle at 5X the recommended use rate. All three died within 24 hours of treatment. The study report concluded that the kittens were stressed from weaning and were not able to tolerate 5X the recommended use rate.

The study is considered **unacceptable** and **cannot be upgraded**. It was terminated prior to completion due to animal welfare considerations.

I. MATERIALS

A. Test Material

Name: Advantage™
Active Ingredients: Imidacloprid
Purity: 9.1% Imidacloprid
Batch Number: MB 9868
Description: Not provided
Storage Conditions: Not provided

Control: Vehicle

B. Administration: dermal

C. Test Animals

Species: Cat, domestic short hair
Source: Not provided
Age: 6 weeks-old
Weight: Mean of ≈ 0.5 kg (males and females) at study initiation
Housing: Two per cage
Environmental Conditions: Not provided
Food and Water: Harlan Teklad Cat Diet and water *ad libitum*
Acclimation Period: Not provided

II. METHODS

A. Dosage and Administration

A total of 24 kittens were assigned to the following four groups based upon gender, age and body weight.

Group 1 - Placebo (Vehicle Minus Active Ingredient) Treated Controls, Males: Use Rate

Group 2 - Placebo (Vehicle Minus Active Ingredient) Treated Controls, Females: Use Rate

Group 3 - Imidacloprid Treated, Males; 5X Use Rate

Group 4 - Imidacloprid Treated, Females; 5X Use Rate

The study report does not state how the 5X use rate was achieved. The Certificate of Assay for the lot of material used in the study (Attachment 3 of the study report) states that 10.0% of

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imidacloprid was present in the sample. Therefore, the 5X use rate must have been achieved by multiple applications of the end-use product. According to Table 2 of the study report, the control animals received a treatment volume of 0.4 ml; the treated animals received 2.0 ml. The study report should have stated where on the animal's body the product was applied.

According to the study protocol (Attachment 1), eight treatments on a weekly basis were planned. However, the study was terminated after the second treatment due to the death of four kittens in the treated group after the first treatment. See Results section for a description of the findings.

B. Observations

The study report states that the animals were observed for clinical signs of toxicity during the course of the study, but not how frequently. They were examined at weekly intervals for signs of dermal irritation.

C. Body Weights

Body weights were suppose to be measured pretreatment, at the time of the second, fourth and sixth treatments and then five days after the last treatment.

D. Hematology and Clinical Chemistry

Pretreatment blood samples were taken two days before treatment for the following hematology and clinical chemistry parameters.

Hematology

White blood cell count (WBC)
Red blood cell count (RBC)
Hemoglobin (HGB)
Mean corpuscular hemoglobin (MCH)
Mean corpuscular volume (MCV)
Mean corpuscular hemoglobin concentration (MCHC)
Platelet count
WBC differential
Hematocrit (HCT)

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Clinical Chemistry

Sodium (Na)
Chloride (Cl)
Blood urea nitrogen (BUN)
Creatinine (Cr)
Creatine kinase (CPK)
Lactate dehydrogenase (LDH)
Albumin (A)
A/G Ratio
Phosphorus ($PO_4$)
Ca/$PO_4$ ratio
Gamma-glutamyl transpeptidase (GGT)
Aspartate aminotransferase (AST)
Alanine aminotransferase (ALT)
Potassium (K)
Carbon dioxide ($CO_2$)
Uric acid
BUN/Cr ratio
Alkaline phosphatase (AP)
Total protein
Globulin (G)
Calcium (Ca)
Cholesterol
Na/K ratio
Bilirubin (total, direct & indirect)

## III. RESULTS

The study report states that at pretreatment the kittens were thin and immature, but did not show critical clinical abnormalities. Therefore, they were included in the study. Four of the 12 kittens, two males and two females, died or were euthanized after the first treatment. One of the males became comatosed within 24 hours of treatment and was euthanized; the other male died within 24 hours post-treatment. One female had muscle tremors and incoordination within 24 hours post-treatment and was euthanized at 72 hours; the other female had similar clinical signs and died at 72 hours. Based on Table 3 of the study report (page 9), the animals which died received the highest dose on a mg/kg basis of the formulation for their respective groups. The males which died received an average of 635 mg/kg, whereas the other four males in the group had an average dose of 311 mg/kg. The dose of the major excipient was 5284 mg/kg in the deceased animals and 2585.8 mg/kg in the surviving males. The females which died received an average of 380.5 mg/kg of the formulation, whereas the other four females received an average dose of 346.3 mg/kg. The dose of the excipient was 3169.5 mg/kg in the deceased females and 2880 mg/kg in the surviving animals. On histological examination, one male had moderate diffuse alveolar edema of the lung; the other male had no lesions. One female had mild diffuse hepatic lipidosis and moderate multifocal suppurative cholangiohepatitis. According to the pathologist's report, the lipidosis was believed to reflect anorexia. The suppurative cholangiohepatitis was assumed to be due to ascending bacterial infection in the liver. The other kitten also had suppurative cholangiohepatitis. Virus isolation was negative for all four kittens.

None of the other animals in either the control or treated groups had clinical signs of toxicity. The control and surviving treated animals gained weight from pretreatment to study termination.

## IV. PROTOCOL REVISIONS

The study was terminated after the second treatment (according to Table 2 of the study report). Three additional kittens were then treated with 5X doses of the vehicle control minus the active ingredient. The kittens expired within 24 hours of treatment with similar symptoms to the other kittens which died. Apparently no necropsy was done on these animals. The study report states that the death of the vehicle-treated kittens substantiated that the major excipient induced the toxicity observed in the Advantage™-treated kittens.

## IV. CONCLUSIONS FROM STUDY REPORT

The study report concludes that immature kittens which are in the transition of being weaned and therefore stressed and depressed might not be able to cope with 5X the recommended treatment use rate. In a telephone conversation with James Schmidl, D.V.M., one of the study authors, on July 31, 1997, he stated that the kittens were stressed after being transported a long distance, were not eating properly when the study was initiated and were not properly conditioned.

## V. STUDY DEFICIENCIES

A. The study report should state how the 5X dose was achieved, i.e., how much material was applied to which sites on the body. In the telephone conversation on July 31, 1997, Dr. Schmidl said all of the test material was applied at the base of the skull, the label recommended method of application.

B. The animals which died after being treated with the vehicle at 5X should have been necropsied for comparison to the necropsy findings on the imidacloprid-treated animals.

C. The study report is unclear about how many treatments were applied. According to Table 2, the surviving animals from the first treatment received a second treatment one week later. However, Table 3 only provides observations following the first treatment.

## VII. COMPLIANCE

The study report states that the study was performed in conformance with the Good Laboratory Practice Standards. A Quality Assurance Statement and a Supplemental Statement of Data Confidentiality Claims are included.

Reviewed by: Virginia A. Dobozy, V.M.D., M.P.H Virginia A Dobozy Date 9/17/97
Reregistration Review Branch I, Health Effects Division (7509C)
Secondary Reviewer: SanYvette Williams-Foy, D.V.M S. A. Williams-Foy Date 9/17/97
Registration Action Branch II, Health Effects Division (7509C)

# DATA EVALUATION REPORT

STUDY TYPE: Domestic Animal Safety Study/Puppies (86-1; OPPTS 870.7200)

DP Barcode: D229579
P. C. CODE: 129099

Submission Code: S511142
Tox. Chem. No.:

TEST MATERIAL (PURITY): Imidacloprid (Advantage™) (9.1%)

SYNONYMS:

CITATION: Schmidl, J. and R. Arther (1996) General Safety Evaluation for Topical Use of Imidacloprid (Advantage™) Spot-On on Puppies. DeSoto Research Facility. Report No. TR-96D-003, July 10, 1996. MRID 44099801. Unpublished.

SPONSOR: Bayer Corporation
Shawnee Mission, Kansas

EXECUTIVE SUMMARY: In a domestic animal safety study (MRID # 44099801), six 7 week-old puppies/sex were treated with Advantage™ (9.1% imidacloprid) at 5X the recommended use rate (2.0 ml if < 10 lbs; 5.0 ml if > 10 lbs) at weekly intervals for eight treatments. Six puppies/sex were treated with the vehicle control at the recommended use rate (0.4 ml if < 10 lbs; 1.0 ml if > 10 lbs) at weekly intervals for eight treatments. There was no evidence of treatment-related toxicity in clinical signs or clinical pathology parameters. All animals gained weight during the study. It was demonstrated that 7 week-old puppies can tolerate a dose of 5X the recommended use rate.

The study is considered **acceptable** and **satisfies the draft guideline requirements (81-6) for a domestic animal safety study.**

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# I. MATERIALS

## A. Test Material

Name: Advantage™
Active Ingredients: Imidacloprid
Purity: 9.1% Imidacloprid
Batch Number: MB9868
Description: Not provided
Storage Conditions: At room temperature and shielded from bright light

Control: Vehicle

## B. Administration: dermal

## C. Test Animals

Species: Dogs, beagles
Source: Not provided
Age: ≈ 7 weeks old at study initiation
Weight: Initial body weight range of 1.4 to 3.1 kg
Housing: Three per cage for the first 28 days of the study and then 3/run for remainder of study
Environmental Conditions: Not provided
Food and Water: Science Diet Growth and water *ad libitum*
Acclimation Period: Not provided

# II. METHODS

## A. Dosage and Administration

A total of 24 puppies were assigned to the following four groups:

Group 1 - 6 males (controls) to receive 8 treatments at 7 day intervals of placebo (minus active ingredient) equivalent to use rate volume

Group 2 - 6 females (controls) to receive 8 treatments at 7 day intervals of placebo (minus active ingredient) equivalent to use rate volume

Group 3 - 6 males to receive 8 treatments at 7 day intervals of formulation at the rate of 5X the labeled use rate volume

Group 4 - 6 females to receive 8 treatments at 7 day intervals of formulation at the rate of 5X the

labeled use rate volume

The use rate is 0.4 ml for dogs weighing under 10 lbs or less and 1.0 ml for those greater than 10 lbs. Treatment volumes were adjusted to increasing weights.

The study report states that the treatment volumes administered on to the skin in the shoulder area were as great as 5.0 ml for those receiving the formulation and up to 1.0 ml for the placebo control groups.

B. Observations

The study report states that the animals were observed daily for clinical signs of toxicity during study. They were examined on weekly intervals for signs of dermal irritation.

C. Body Weights

The puppies were weighed pretreatment and at 14 day intervals during the study.

D. Hematology and Clinical Chemistry

Blood samples were taken pretreatment, 3 days after the second treatment and one week after the last treatment. The following hematology and clinical chemistry parameters were measured.
Hematology

White blood cell count (WBC)
Red blood cell count (RBC)
Hemoglobin (HGB)
Mean corpuscular hemoglobin (MCH)
Mean corpuscular volume (MCV)
Mean corpuscular hemoglobin concentration (MCHC)
Platelet count
WBC differential
Hematocrit (HCT)

Clinical Chemistry

Sodium (Na)
Chloride (Cl)
Blood urea nitrogen (BUN)
Creatinine (Cr)
Creatine kinase (CPK)
Lactate dehydrogenase (LDH)
Albumin (A)
A/G Ratio
Phosphorus ($PO_4$)
$Ca/PO_4$ ratio
Gamma-glutamyl transpeptidase (GGT)
Aspartate aminotransferase (AST)
Alanine aminotransferase (ALT)
Potassium (K)
Carbon dioxide ($CO_2$)
Uric acid
BUN/Cr ratio
Alkaline phosphatase (AP)
Total protein
Globulin (G)
Calcium (Ca)
Cholesterol
Na/K ratio
Bilirubin (total, direct & indirect)

## III. RESULTS

The average mg/kg dose applied at each treatment for males and females is presented in Table 1 below. The high mg/kg dose for the seventh and eighth treatments for Group 3 resulted because three animals in the group weighed over 10 pounds and thus were treated with 5 ml of the product (5 times the recommended dose of 1 ml).

Table 1: Dose Received by Puppies Treated with Advantage™[a]

| | Treatment | Mg/Kg Dose | Mg/Kg Range |
|---|---|---|---|
| Group 3 | 1st and 2nd | 87.8 | 67 - 111 |
| | 3rd and 4th | 80.6 | 61 - 105 |
| | 5th and 6th | 55.0 | 43 - 65 |
| | 7th and 8th | 130.0 | 50 - 213 |
| Group 4 | 1st and 2nd | 111.3 | 95 - 133 |
| | 3rd and 4th | 102.2 | 91 - 125 |
| | 5th and 6th | 72.8 | 61 - 95 |
| | 7th and 8th | 56.3 | 48 - 67 |

a Calculations performed by reviewer based on data in Table 3 (page 12) of the study report.

The study report indicates that there were no clinical signs of toxicity or dermal irritation. All animals gained weight over the course of the study. The study report states that there were no

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clinically significant trends in the hematology and clinical chemistry parameters.

## IV. CONCLUSIONS FROM STUDY REPORT

The study report concluded that there was no evidence of adverse effects in puppies as young as six weeks when they received dermal treatments of Imidacloprid 9.1% at 5X the use rate at seven day intervals for 8 consecutive weeks. Note: pups were born on March 16-19, 1996. The first treatment was on May 3, 1996. Therefore, the puppies were 48-51 days old, closer to 7 weeks-than 6 weeks-old.

## V. STUDY DEFICIENCIES

A. No data on clinical signs were submitted. The study protocol in Attachment 1 contains tables for recording various clinical signs. They should have been included with the study, even if all the animals appeared normal. After a telephone conversation with James Schmidl, D.V.M., one of the study authors on July 31, 1997, the raw data were forwarded directly to the reviewer. On review of these data, none of the control or treated animals had evidence of clinical signs of toxicity.

B. The timing of the hematology and clinical chemistry evaluations was not optimal for detecting an acute effect. The draft Domestic Animal Safety Guidelines recommend that this testing be conducted 24 hours post-treatment. In this study, the interim testing was done at three days after the second treatment and two days after the final treatment. Testing should have been after the first treatment. However, there was no evidence of a treatment-related effect on the parameters. Review of these data would have been facilitated by preparation of tables of mean values.

## VI. COMPLIANCE

The study report states that the study was performed in conformance with the Good Laboratory Practice Standards. A Quality Assurance Statement and a Statement of No Data Confidentiality Claims are included.

Reviewed by: Virginia A. Dobozy, V.M.D., M.P.H. Virginia A Dobozy Date 9/17/97
Reregistration Review Branch I, Health Effects Division (7509C)
Secondary Reviewer: SanYvette Williams-Foy, D.V.M. S. A Williams-Foy Date 9/17/97
Registration Action Branch II, Health Effects Division (7509C)

# DATA EVALUATION REPORT

STUDY TYPE: Domestic Animal Safety Study/Kittens (86-1; OPPTS 870.7200)

DP Barcode: D232036
P. C. CODE: 129099
Submission Code: S515745
Tox. Chem. No.:

TEST MATERIAL (PURITY): Imidacloprid (Advantage™) (9.1%)

SYNONYMS:

CITATION: Schmidl, J. and R. Arther (1996) General Safety Evaluation for Topical Use of Imidacloprid (Advantage™) Spot-On on Kittens Eight Weeks of Age. DeSoto Research Facility. Report No. TR-96F-006, August 30, 1996. MRID 44157302. Unpublished.

SPONSOR: Bayer Corporation
Shawnee Mission, Kansas

EXECUTIVE SUMMARY: In a domestic animal safety study (MRID # 44157302), six 8 week-old kittens/sex were treated with Advantage™ (9.1% imidacloprid) at 5X the recommended use rate (2.0 ml) at weekly intervals for eight treatments. Six kittens/sex were treated with the vehicle control at the recommended use rate (0.4 ml) at weekly intervals for eight treatments. There was no evidence of treatment-related toxicity in clinical signs or clinical pathology parameters. All animals gained weight during the study. It was demonstrated that 8 week-old kittens can tolerate a dose of 5X the recommended use rate.

The study is considered **acceptable** and **satisfies the draft guideline requirements (81-6) for a domestic animal safety study.**

# I. MATERIALS

## A. Test Material

Name: Advantage™
Active Ingredients: Imidacloprid
Purity: 9.1% Imidacloprid
Batch Number: 418004
Description: Not provided
Storage Conditions: Not provided

Control: Vehicle

## B. Administration: dermal

## C. Test Animals

Species: Cat, domestic short hair
Source: Not provided
Age: 8 weeks-old at study initiation
Weight: Mean of ≈ 1.0 kg (males and females) at study initiation
Housing: Two per cage
Environmental Conditions: Not provided
Food and Water: Science Diet and water *ad libitum*
Acclimation Period: Not provided

# II. METHODS

## A. Dosage and Administration

A total of 24 kittens were assigned to the following four groups based upon gender, age and body weight.

Group 1 - 6 males (controls) to receive 8 treatments at 7 day intervals of placebo (minus active ingredient) equivalent to use rate volume

Group 2 - 6 females (controls) to receive 8 treatments at 7 day intervals of placebo (minus active ingredient) equivalent to use rate volume

Group 3 - 6 males to receive 8 treatments at 7 day intervals of formulation at the rate of 5X the labeled use rate volume

Group 4 - 6 females to receive 8 treatments at 7 day intervals of formulation at the rate of 5X the

labeled use rate volume

The use rate is 0.4 ml for cats weighing under 9 lbs. and 0.8 ml for those greater than 9 lbs.

The study report does not state how the 5X use rate was achieved. The Certificate of Assay for the lot of material used in the study (found in Attachment 2 of the study report) states that 10.0% of imidacloprid was present in the sample. Therefore, the 5X use rate must have been achieved by multiple applications of the end-use product. According to Table 2 of the study report, the control animals received a treatment volume of 0.4 ml; the treated animals received 2.0 ml. The study report should have stated how much material was applied to individual sites on the animal's body.

B. Observations

The study report states that the animals were observed daily for clinical signs of toxicity during study. They were examined on weekly intervals for signs of dermal irritation.

C. Body Weights

The kittens were weighed pretreatment and at 14 day intervals during the study.

D. Hematology and Clinical Chemistry

Blood samples were taken pretreatment, 3 days after the second treatment and two weeks after the last treatment. The following hematology and clinical chemistry parameters were measured.
Hematology

White blood cell count (WBC)
Red blood cell count (RBC)
Hemoglobin (HGB)
Mean corpuscular hemoglobin (MCH)
Mean corpuscular volume (MCV)
Mean corpuscular hemoglobin concentration (MCHC)
Platelet count
WBC differential
Hematocrit (HCT)

Clinical Chemistry

Sodium (Na)
Chloride (Cl)
Blood urea nitrogen (BUN)
Creatinine (Cr)
Creatine kinase (CPK)
Lactate dehydrogenase (LDH)
Albumin (A)
A/G Ratio
Phosphorus ($PO_4$)
$Ca/PO_4$ ratio
Gamma-glutamyl transpeptidase (GGT)
Aspartate aminotransferase (AST)
Alanine aminotransferase (ALT)
Potassium (K)
Carbon dioxide ($CO_2$)
Uric acid
BUN/Cr ratio
Alkaline phosphatase (AP)
Total protein
Globulin (G)
Calcium (Ca)
Cholesterol
Na/K ratio
Bilirubin (total, direct & indirect)

## III. RESULTS

The average mg/kg dose applied at each treatment for males and females is presented in Table 1.

Table 1: Dose Received by Kittens Treated with 2.0 Ml Advantage™[a]

| | Treatment | Mg/Kg Dose | Mg/Kg Range |
|---|---|---|---|
| Group 3 | 1st and 2nd | 243.0 | 211 - 271 |
| | 3rd and 4th | 199.8 | 180 - 225 |
| | 5th and 6th | 143.8 | 132 - 165 |
| | 7th and 8th | 113.2 | 99 - 132 |
| Group 4 | 1st and 2nd | 229.5 | 211 - 241 |
| | 3rd and 4th | 192.0 | 180 - 198 |
| | 5th and 6th | 144.7 | 141 - 152 |
| | 7th and 8th | 121.7 | 110 - 132 |

a Calculations performed by reviewer based on data in Table 3 (page 11) of the study report.

The study report states that there were no clinical signs of toxicity or dermal irritation. All animals gained weight over the course of the study. The study report states that there were no clinically significant trends in the hematology and clinical chemistry parameters.

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## IV. CONCLUSIONS FROM STUDY REPORT

The study report concluded that there was no evidence of adverse effects in kittens as young as eight weeks when they received dermal treatments of Imidacloprid 9.1% at 5X the use rate for 8 consecutive weeks.Note: the kittens were born on 4/24 - 4/30/96. The first treatment was 6/25/96. Therefore, they would have been 56-62 days old at the time of the first treatment.

## V. STUDY DEFICIENCIES

A. The study report should state how the 5X dose was achieved, i.e., how much material was applied to which sites on the body. In a telephone conversation with James Schmidl, D.V.M., one of the study authors on July 31, 1997, he said all the test material was applied at the base of the skull, as the label directions recommend.

B. No data on clinical signs were submitted. The study protocol in Attachment 1 contains tables for recording various clinical signs. They should have been included with the study, even if all the animals appeared normal. After the telephone conversation with Dr. Schmidl, the raw data were sent to the reviewer. On review of these data, there is no indication that either control or treated animals had any clinical signs.

C. The timing of the hematology and clinical chemistry evaluations was not optimal for detecting an acute effect. The draft Domestic Animal Safety Guidelines recommend that this testing be conducted 24 hours post-treatment. In this study, the interim testing was done at three days after the second treatment and two days after the final treatment. Testing should have been done after the first treatment. However, there was no evidence of a treatment-related effect on the parameters. Review of these data would have been facilitated by preparation of tables of mean values.

D. The exact product formulation, including the concentration of the major excipient, should have been included. Severe clinical signs, including death, were observed in a study on 6 week-old kittens (MRID #44157301) in which the toxicity was attributed to the major excipient. Dr. Schmidl stated that the formulation used in both this study and the present one was identical to that currently registered. This information is also included in a letter to the reviewer that accompanied the raw data for clinical signs.

## VI. COMPLIANCE

The study report states that the study was performed in conformance with the Good Laboratory Practice Standards. A Quality Assurance Statement and a Statement of No Data Confidentiality Claims are included.

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